

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph at page 37, line 28 to page 38, line 14 with the following amended paragraph:

To assess whether ADD-1 regulates the expression of the hPPAR γ 3 mRNA, we cotransfected either an expression plasmid coding for ADD-1 together with the pGL3 γ 3p800 luciferase reporter vector. When Hep G2 cells were cotransfected with the ADD-1 expression vector, the luciferase activity was increased at least 4-fold relative to the basal ~~activity~~ activity of the hPPAR γ 3 promoter. As a control we transfected the pGL31p3000 and pGL3 γ 2p1000 expression plasmids, which contain respectively 3 kb and 1 kb of the human PPAR γ 1 and 72 promoter (Fajas *et al.*, *J. Biol. Chem.* 272:18779-18789 (1997)). Activity of these two reporter constructs was unaffected when the ADD-1 expression vector was cotransfected, suggesting that the effect on the PPAR γ 3 promoter was specific. To unequivocally demonstrate that it is through binding to the PPAR γ 3-E-box that ADD-1 stimulates the activity of the hPPAR γ 3 promoter, we substituted three bases in the PPAR γ 3-E-box (from ATTCATGTGACAT (SEQ ID NO: 41) to ATTCATGCATCAT (SEQ ID NO: 42)) to generate the pGL3 γ 3p800-E-boxmut reporter plasmid. Cotransfected ADD-1 is unable to stimulate the mutated pGL3 γ 3p800-E-boxmut reporter vector in Hep G2 cells, whereas in the same experiment, the wild-type promoter is induced by 4-fold. Qualitatively similar results were obtained when an expression vector for mouse SREBP-1a was used.

Please replace the paragraph at page 46, lines 16 – 29 with the following amended paragraph:

The Marathon cDNA amplification kit (CLONTECH) was used to obtain a library of adaptor-ligated double-stranded cDNA from human adipose tissue. 1 μ g of poly(A)+ RNA was used as a template for the first strand synthesis, with the 52-mer CDS primer and 100 units of the MMLV reverse transcriptase in a total volume of 10 μ l. Synthesis was carried out at 42 °C for 1 h. Next, the second strand was ~~synthesized~~ synthesized at 16 °C for 90 min in a total volume of 80 μ l containing the enzyme mixture (RNase H, Escherichia coli DNA polymerase I, and E. coli DNA ligase), the second strand buffer, the dNTP mix, and the first strand reaction. cDNA ends were then made blunt by adding to the reaction 10 units of T4 DNA polymerase and incubating at 16 °C for 45 min. The double-stranded cDNA was phenol/chloroform extracted, ethanol precipitated, and resuspended in 10 μ l of water. Half of this volume was used to ligate the adaptor to the cDNA ends (adaptor sequence (SEQ ID NO: 60) CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCCGGGCAGGT) in a total

volume of 10 µl using 1 unit of T4 DNA ligase. The ligation reaction was incubated 16 h at 16°C. The resulting cDNA library was diluted to a final concentration of 0.1 mg/ml.

Please replace the paragraph at page 48, line 14 to page 49, line 3 with the following amended paragraph:

RNA preparation of total cellular RNA was performed as described previously (37). The absolute mRNA concentration of the differentially spliced PPAR γ variants was measured by reverse transcription reaction followed by competitive polymerase chain reaction (RT-competitive PCR) in the presence of known amounts of competitor DNA yielding amplicons of different size allowing the separation and the quantification of the PCR products. The competitor was constructed by deletion of a 74-bp fragment (nucleotides +433 to +507 by HindIII digestion) of PPAR γ 1 cloned into pBluescript KS+, yielding pBSCompPPAR γ . Working solution of the competitor was prepared by in vitro transcription followed by serial dilution in 10 mM Tris-HCl (pH 8.3), 1 mM EDTA buffer. For RT-competitive PCR, the antisense primer hybridized to the 3'-end of exon 3 (gammaAS:5'-GCATTATGAGCATCCCCAC-3', nt +600 to +620 SEQ ID NO: 27) and the sense primer to exon 1 (gammaS:5'-TCTCTCCGTAATGGAAGACC-3', nt +146 to +165 SEQ ID NO: 28) or to the B exon (gamma2S:5'-GCGATTCCTTCACTGATAC-3', nt +41 to +59 SEQ ID NO: 29). Therefore, the same competitor served to measure either total PPAR γ mRNAs (γ 1 + γ 2; with primers gamma AS and gamma S) or, specifically, PPAR γ 2 mRNA (with primers gamma AS and gamma 2S). The gamma AS/gamma S primer pair gave PCR products of 474 and 400 bp for the PPAR γ mRNAs and competitor, respectively. The primer pair gammaAS/gamma2S gave 580 bp for PPAR γ 2 mRNA and 506 bp for the competitor. For analysis of the PCR products, the sense primers gamma S and gamma2S were 5'-end labeled with the fluorescent dye Cy-5 (Eurogentec, Belgium).

Please replace the paragraph at page 51, line 29 to page 52, line 14 with the following amended paragraph:

Site-directed mutagenesis of the E-box in the PPAR γ 3 promoter was performed by splicing overlapping ends polymerase chain reaction (Ho, *et al.*, *Gene* 77:51-59 (1989)), using the oligo pairs LF-106/LF-60 and LF-107/LF-68, to generate the plasmid pGL3 γ 3p800-E-boxmut. This changed the three bases underlined in the sequence 5'-ATTCATGTGACAT-3' to 5'-ATTCATGCATCAT-3' (SEQ ID NOS: 41 and 42, respectively). The J3-TK-LUC (Vu-Dac *et al.*, 1995) and ACO-TK-LUC (Osumi *et al.*, *Biophys. Res. Commun.* 175:866-871 (1991)) luciferase reporter vectors and the expression vectors encoding for ADD-1, a

dominant negative form of ADD-1, and SREBP-1a (Tontonoz *et al.*, *Mol. Cell. Biol.* 13:4753-4759 (1993)); Yokoyama *et al.*, *Cell* 75:187-197 (1993)) were described before. Transfections, luciferase and β -galactosidase assays were generally performed as described previously (Schoonjans *et al.*, *J. Biol. Chem.* 270:19269-19276 (1995)). To analyze the effect of cholesterol depletion in transfection experiments, the cells were divided in two pools after transfection. Half of the transfected cells were incubated with delipidated medium, whereas the other half of the cells were incubated with the same medium supplemented with a mixture of 10 μ M cholesterol and 1 μ M of 25-hydroxycholesterol.

Please replace the paragraph at page 52, lines 18 – 30 with the following amended paragraph:

haPPAR γ (39), hPPAR γ 2, and mRXR α (40) proteins were synthesized in vitro in rabbit reticulocyte lysate (Promega). Molecular weights and quality of the in vitro translated proteins were verified by SDS-PAGE. PPAR (2 μ l) and/or RXR (2 μ l) were incubated for 15 min on ice in a total volume of 20 μ l with 1-ng probe, 2.5 μ g of poly(dI-dC) and 1 μ g of herring sperm DNA in binding buffer (10 mM Tris-HCl pH 7.9, 40 mM KCl, 10% glycerol, 0.05% Nonidet P-40, and 1 mM dithiothreitol). For competition experiments, increasing amounts (from 10- to 200-fold molar excess) of cold oligonucleotide (AII-J-PPRE, 5'-GATCCTTCAACCTTTACCCTGGTAGA-3' (41) SEQ ID NO: 24; acyl-CoA oxidase (ACO)-PPRE, 5'-GATCCCGAACGTGACCTTTGTCCTGGTCCC-3' (42) SEQ ID NO: 25; or LPL-PPRE, 5'-GATCCGTCTGCCCTTTCCCCCTCTTCA-3' (23) SEQ ID NO: 26; were included just before adding T4-PNK end-labeled AII-J-PPRE oligonucleotide. DNA-protein complexes were separated by electrophoresis on a 4% polyacrylamide gel in 0.25 x TBE buffer at 4°C (43).